Claims:

1. A method of screening for an agent to determine its usefulness in treating insulin resistance, the method comprising:

(a) establishing a paradigm in which at least one protein is differentially expressed in relevant tissue from, or representative of, subjects having differential levels of insulin sensitivity;

(b) obtaining a sample of relevant tissue taken from, or representative of, an insulin resistant subject, who or which has been treated with the agent being screened;

(c) determining the presence, absence or degree of expression of the differentially expressed protein or proteins in the tissue from, or representative of, the treated subject; and,

(d) selecting or rejecting the agent according to the extent to which it changes the expression, activity or amount of the differentially expressed protein or proteins in the treated insulin resistant subject.

2. The method of claim 1, wherein the agent is selected if it changes the expression of the differentially expressed protein or proteins towards that of a normal subject or more insulin sensitive subject.

3. The method of claim 2, wherein the agent is selected if it converts the expression of the protein or proteins to that of a normal or more insulin sensitive subject.

Sub A'

4. The method of any one of claims 1 to 3, ~~wherein~~ in the paradigm, the subjects ~~having~~ differential levels of insulin sensitivity comprise normal subjects and insulin resistant subjects.

5. The method of any one of claims 1 to 3, wherein in the paradigm the subjects having differential levels of insulin sensitivity comprise normal subjects and abnormally insulin sensitive subjects.

6. The method of claim 5, wherein the abnormally insulin sensitive subjects have acquired higher than normal sensitivity by exercise.

7. The method of any one of the preceding claims, wherein the relevant tissue is liver, skeletal muscle, white or brown adipose tissue.

8. The method of any one of the preceding claims, wherein in the paradigm, the subjects having differential levels of protein expression comprise:

(a) comparatively insulin sensitive subjects and insulin resistant subjects; and,

(b) insulin resistant subjects which have not been treated with the agent and insulin resistant subjects which have been treated with the agent.

9. The method of claim 8, wherein the differential levels of protein expression are not observed in the comparatively insulin sensitive subjects who have and have not been treated with the agent.

10. The method of any one of the preceding claims, wherein in the paradigm, the subjects having differential levels of protein expression comprise:

(a) comparatively insulin sensitive subjects who have and have not been treated with the agent; and,

(b) insulin resistant subjects who have and have not been treated with the agent.

11. The method of claim 10, wherein the differential levels of protein expression are not observed in comparatively insulin sensitive subjects and insulin resistant subjects, both groups of subjects being untreated with the agent.

12. The method of any one of claims 8 to 11, wherein the comparatively insulin sensitive subjects are normal subjects or abnormally insulin sensitive subjects.

13. The method of claim 12, wherein the abnormally insulin sensitive subjects have acquired higher than normal sensitivity by exercise.

14. The method of any one of the preceding claims, wherein in the paradigm the insulin-resistant subjects are animals which are insulin-resistant as a result of genetic mutation, and the normal subjects are normal control animals.

15. The method of claim 14, wherein the normal control animals are insulin sensitive littermates of the genetically mutated animals.

16. The method of any one of the preceding claims, wherein the paradigm is established in tissue from, or representative of, animals which are insulin-resistant as a result of diet, and the normal subjects are normal control animals.

17. The method of any one of the preceding claims, wherein in the paradigm the normal and insulin resistant subjects are animals which are insulin-sensitive on a natural diet, but develop insulin resistance when given an unnatural, laboratory diet.

18. The method of any one of the preceding claims, wherein in the paradigm the treatment to increase the level of insulin sensitivity comprises treatment with an insulin-sensitising drug.

19. The method of claim 18, wherein the insulin-sensitising drug is a thiazolidinedione.

20. The method of claim 19, wherein the thiazolidinedione is rosiglitazone (BRL 49653).

21. The method of claim 18, wherein the insulin-sensitising drug is a non-thiazolidinedione which is (a) an agonist or partial agonist of the PPAR gamma nuclear receptor, (b) a b_3-adrenoceptor agonist or (c) a leptin or leptin fragment.

S4607

22. The method of any one of the preceding claims, wherein in the paradigm the treatment to increase the level of insulin sensitivity comprises dietary restriction and/or exercise.

23. The method of any one of the preceding claims, wherein the sample obtained is taken from or is representative of a subject suffering from non-insulin dependent diabetes.

24. The method of any one of the claims 1 to 22, wherein the sample is taken from or is representative of a subject suffering from polycystic ovary syndrome, syndrome X, insulin resistance syndrome or type I diabetes.

25. The method of any one of the preceding claims, wherein the paradigm is established by two-dimensional

gel electrophoresis carried out on the relevant tissue or a protein-containing extract thereof.

26. The method of any one of the preceeding claims, wherein the expression of the differentially expressed protein is determined by two-dimensional gel electrophoresis carried out on the sample or a protein-containing extract thereof.

27. The method of any one of the preceding claims, further comprising the step of isolating a differentially expressed protein identified in the method.

28. The method of claim 27, further comprising the step of characterising the isolated protein.

Sub a8

29. The method of any one of the preceding claims wherein the differentially expressed protein or proteins comprises one or more of LOM16, LOM17, LOM18, LOMT19, LOM20, LOMT21, LOMT22, LOMT23, LOMT24, LOMT25, LOMT26, LOM27, LOM28, LOM29 or LSEM30, MOM31, MOM32, MOM33, MOMT34, MOMT35, MOM36, WOMT37, WOM38, WOMT39, WOM40, WOM41, WOMT42, WOM43, WOM46, WOM47, WOMT48, WOMT49, WOMT50, WOM51 to 55, WOM57 to 64, WSEM65, BOM66, BOM67, BOMT68, BOM69 to 75, BOMT76 or BOM77.

30. The method of claim 28, further comprising using the protein in an assay for specific binding partners of the protein.

31. The method of claim 28, further comprising using the protein in an assay to screen for agonists or antagonists of the protein.

Sub a9

~~32. The method of any one of claims 1 to 31, wherein the~~

agents or proteins are screened using a high throughput screening method.

33. A method of making a pharmaceutical composition which comprises having identified an agent using the method of any one of claims 1 to 32, the further step of manufacturing the agent and formulating it with an acceptable carrier to provide the pharmaceutical composition.

34. A protein for use in a method of medical treatment, wherein the protein is selected from LOM16, LOM17, LOM18, LOMT19, LOM20, LOMT21, LOMT22, LOMT23, LOMT24, LOMT25, LOMT26, LOM27, LOM28, LOM29 or LSEM30, MOM31, MOM32, MOM33, MOMT34, MOMT35, MOM36, WOMT37, WOM38, WOMT39, WOM40, WOM41, WOMT42, WOM43, WOM46, WOM47, WOMT48, WOMT49, WOMT50, WOM51 to 55, WOM57 to 64, WSEM65, BOM66, BOM67, BOMT68, BOM69 to 75, BOMT76 or BOM77.

35. Use of an agent identified by the method of any one of claims 1 to 32 for the preparation of a medicament for the treatment of a condition characterised by insulin resistance.

36. The use of claim 35, wherein the condition is:

(a) a pre-diabetic condition with respect to, non-insulin dependent diabetes (type 2 diabetes);

(b) type 2 diabetes, polycystic ovary syndrome, syndrome X or insulin resistance syndrome.

37. The use of claim 35 or claim 36, wherein the agent is a protein is selected from LOM16, LOM17, LOM18, LOMT19, LOM20, LOMT21, LOMT22, LOMT23, LOMT24, LOMT25, LOMT26, LOM27, LOM28, LOM29 or LSEM30, MOM31, MOM32, MOM33, MOMT34, MOMT35, MOM36, WOMT37, WOM38, WOMT39,

WOM40, WOM41, WOMT42, WOM43, WOM46, WOM47, WOMT48, WOMT49, WOMT50, WOM51 to 55, WOM57 to 64, WSEM65, BOM66, BOM67, BOMT68, BOM69 to 75, BOMT76 or BOM77.

Sub Q 10

38. A method of treating a condition characterised by insulin resistance in a patient, the method comprising administering a therapeutically or prophylactically effective amount of such an agent identified by a method of any one of claim 1 to 32 to the patient.

39. A method of determining the nature or degree of insulin resistance in a sample of relevant tissue from a human or animal subject, which comprises:

(a) establishing a paradigm in which at least one protein is differentially expressed in relevant tissue from, or representative of, subjects having differential levels of insulin sensitivity,

(b) obtaining a sample of the tissue and

(c) determining the presence, absence or degree of expression of the differentially expressed protein or proteins in the sample, and

(d) relating the determination to the nature or degree of the insulin resistance by reference to a previous correlation between such a determination and clinical information.

40. The method of claim 39, wherein in the paradigm at least four proteins are differentially expressed, providing a multi-protein fingerprint of the nature or degree of the insulin resistance.

Sub Q 11

41. The method of claim 39 or 40, wherein in the paradigm the subjects having differential levels of insulin sensitivity comprise normal subjects and insulin resistant subjects.

42. The method of any one of claims 39 to 41, wherein the subjects having differential levels of insulin sensitivity comprise normal subjects and subjects having abnormally high insulin sensitivity.

43. The method of any one of claims 39 to 42, which further comprises determining an effective therapy for treating the abnormality.

44. The method of any one of claims 39 to 43, wherein the sample is taken from a patient undergoing treatment for the insulin resistance and wherein the method further comprises monitoring the treatment.

45. A protein which is differentially expressed in relevant tissue from, or representative of subjects having differential levels of insulin sensitivity and which is obtainable by the method of two-dimensional gel electrophoresis carried out on said tissue or a protein-containing extract thereof, the method comprising:

(a) providing non-linear immobilized pH gradient (IPG) strips of acrylamide polymer 3 mm x 180 mm;

(b) rehydrating the IPG strips in a cassette containing 25 ml. of an aqueous solution of urea (8M), 3-[(cholamidopropyl)dimethylammonio]-1-propanesulphonate (CHAPS, 2% w/v), dithioerythritol (DTE, 10mM), mixture of acids and bases of pH 3.5 to 10 (2% w/v) and a trace of Bromophenol Blue;

(c) emptying the cassette of liquid, transferring the strips to an electrophoretic tray fitted with humid electrode wicks, electrodes and sample cups, covering the strips and cups with low viscosity paraffin oil;

(d) applying 200 micrograms of an aqueous solution of dried, powdered material of the relevant body tissue in urea (8M), CHAPS (4% w/v), Tris (40 mM), DTE (65 mM),

SDS (0.05% w/v) and a trace of Bromophenol Blue to the sample cups, at the cathodic end of the IPG strips;

(e) carrying out isoelectric focusing on the gel at a voltage which increases linearly from 300 to 3500 V during 3 hours, followed by another 3 hours at 3500 V, and thereafter at 5000V for a time effective to enable the proteins to migrate in the strips to their pI-dependent final positions;

(f) equilibrating the strips within the tray with 100 ml of an aqueous solution containing Tris-HCl (50 mM) pH 6.8, urea (6M), glycerol (30% v/v), SDS (2% w/v) and DTE (2% w/v) for 12 minutes;

(g) replacing this solution by 100 ml. of an aqueous solution containing Tris-HCl (50 mM) pH 6.8, urea (6M), glycerol (30% v/v), SDS (2% w/v), iodoacetamide(2.5% w/v) and a trace of Bromophenol Blue for 5 minutes;

(h) providing a vertical gradient slab gel 160 x 200 x 1.5 mm of acrylamide/piperazine-diacrylyl cross-linker(9-16%T/2.6%C), polymerised in the presence of TEMED (0.5% w/v), ammonium persulphate (0.1% w/v) and sodium thiosulphate (5 mM), in Tris-HCl (0.375M) pH 8.8 as leading buffer;

(i) over-layering the gel with sec-butanol for about 2 hours, removing the overlay and replacing it with water;

(j) cutting the IPG gel strips to a size suitable for the second dimensional electrophoresis, removing 6 mm from the anode end and 14 mm from the cathode end;

(k) over-layering the slab gel with an aqueous solution of agarose (0.5% w/v) and Tris-glycine-SDS (25 mM-198 mM-0.1% w/v) as leading buffer, heated to 70°C and loading the IPG gel strips onto the slab gel through this over-layered solution;

(l) running the second dimensional electrophoresis

at a constant current of 40 mA at 8-12°C for 5 hours; and

(m) washing the gel.

46. A differentially expressed protein of claim 45 as obtainable from mouse liver cells of obese, insulin resistant mice, which may have been treated with rosiglitazone, and designated herein LOM16, LOM17, LOM18, LOMT19, LOM20, LOMT21, LOMT22, LOMT23, LOMT24, LOMT25, LOMT26, LOM27, LOM28, LOM29 or LSEM30.

47. A differentially expressed protein of claim 45 as obtainable from skeletal muscle cells of obese, insulin resistant mice, which may have been treated with rosiglitazone, and designated herein MOM31, MOM32, MOM33, MOMT34, MOMT35 or MOM36.

48. A differentially expressed protein of claim 45 as obtainable from white adipose tissue of obese, insulin resistant mice, which may have been treated with rosiglitazone, and designated herein WOMT37, WOM38, WOMT39, WOM40, WOM41, WOMT42, WOM43, WOM46, WOM47, WOMT48, WOMT49, WOMT50, WOM51 to 55, WOM57 to 64 or WSEM65.

49. A differentially expressed protein according to claim 45 as obtainable from brown adipose tissue of obese, insulin resistant mice, which may have been treated with rosiglitazone, and designated herein BOM66, BOM67, BOMT68, BOM69 to 75, BOMT76 or BOM77.

50. A differentially expressed protein having one or more of the identifying characteristics set out in Table 1 to 4.

51. The differentially expressed protein of claim 50,

wherein the identifying characteristics are pI and Mw.

sub a'12

52. A method whereby the pattern of differentially expressed proteins in a tissue sample or body fluid sample of an individual with insulin resistance is used to predict the most appropriate and effective therapy the alleviate the insulin resistance and to monitor the success of that treatment.

add a'13